Insect Biochem. Vol. 17, No. 6, pp. 783–792, 1987
Printed in Great Britain

0020-1790/87 $3.00 + 0.00
Pergamon Journals Ltd

REGULATION OF PROTEIN SYNTHESIS DURING EGG DEVELOPMENT OF THE PARASITIC WASP, *MICROPLITIS CROCEIPES* (CRESSON) (BRACONIDAE)

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(*Received* 6 *October* 1986; *revised and accepted* 5 *January* 1987)

Abstract—Regulation of protein synthesis was studied in *Microplitis croceipes* (Cresson) during oogenesis and after oviposition in its host, *Heliothis zea* (Boddie). Oocytes were dissected from ovaries of *M croceipes* during various stages of development and from second instar host larvae at various times after egg oviposition. Uridine incorporation into RNA was highest in oocytes in the ovarian egg tube, then declined in oocytes present in the egg reservoir and calyx of the ovary. During blastoderm formation, 4–6 hr after the egg was oviposited into the host, uridine was still incorporated at low levels. At 18–24 hr after oviposition (14–18 hr after blastoderm formation), uridine incorporate returned to the level observed in eggs in the ovarian reservoir. Protein synthesis, measured by [^{3}H]leucine and [^{35}S]methionine incorporation was continuous at all stages of development and was most rapid 14–20 hr after blastoderm formation when RNA synthesis was highest. Patterns of polypeptide synthesis as analyzed by one-dimensional SDS–PAGE changed quantitatively but this method did not reveal any new polypeptides in oocytes in various stages of development or during early and late embryogenesis. However, when polypeptide patterns of eggs were compared on two-dimensional gels during embryogenesis, synthesis of new polypeptides was evident after cellularization of the blastoderm (4–6 hr after oviposition). These changes in synthesis after blastoderm formation may be the result of transcription and subsequent translation of embryonic mRNA.

Key Word Index: *Microplitis croceipes*, *Heliothis zea*, oogenesis, embryogenesis, hemolymph, protein synthesis, two-dimensional electrophoresis

INTRODUCTION

Eggs of hymenopteran endoparasitoids differ from those of free-living insects in several aspects. Eggs of most endoparasitoids are thin-walled, contain little yolk when laid, and increase in volume many times after being deposited. Based on these observations it has generally been assumed that the maturing egg takes up hemolymph components and ions freely through the egg membranes (Fisher, 1971). In the endoparasitoid *Microplitis croceipes* (Cresson), however, maturing eggs were not observed to take up ^{125}I-labeled host hemolymph proteins either *in vivo* or *in vitro* (Ferkovich and Dillard, 1986). The parasitoid egg apparently relies on its own protein synthetic machinery for growth because the eggs readily took up labeled amino acids and synthesized proteins immediately after oviposition and throughout early and late embryogenesis. The trophaminon surrounding the embryo may facilitate this function (Ivanova-Kasas, 1972). Some qualitative differences were evident on one-dimensional SDS gels between eggs in early and late stages of embryogenesis; however, the overall pattern of synthesized proteins remained fairly constant.

A review of the literature offers several examples in *Drosophila melanogaster* (Meigen) of correlating stages of egg development with electrophoretic patterns. Roberts and Graziosi (1977) demonstrated one-dimensional SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) patterns from 1 to 3 hr of development. Although the polypeptides changed quantitatively with time, qualitative differences were not detected. Constant polypeptide profiles also were observed by Savoini *et al.* (1981) for *D. melanogaster* (Meigen) eggs, but observations were extended to 8 hr of development and two-dimensional PAGE was used to resove 400 different polypeptides. In contrast, another study (Trumbly and Jarry, 1983) using similar methods and materials reported a change in the rate of synthesis of 26% of the 261 polypeptides scored.

Production of constant SDS–PAGE profiles during embryogenesis has been explained as the result of stable maternal RNA originating in the nurse cells during oogenesis. Transcription of zygotic RNA is therefore not required for nuclear division, determination, or other events in the egg. Only after cellularization of the blastoderm would translation of the zygotic genome take place. Support for this concept can be seen in the autoradiographic study by Zalokar (1976). Autoradiographic techniques gave substantial evidence for uridine incorporation only after the blastoderm stage.

The purpose of this study was to determine if the initiation of protein synthesis in eggs of *M. croceipes* depends on oviposition into the host, or whether it is a continuation of the protein synthesis within the ovaries of adult females.

MATERIALS AND METHODS

Host and parasitoid colony maintenance

The host species *Heliothis zea*, and the parasitoid *M. croceipes*, were reared as described by Ferkovich and Dillard (1986).

Dissection of host larvae

Parasitized second instar *H. zea* larvae were anesthetized and surface sterilized by a 5 min immersion in 70% ethanol, blotted to remove excess water and alcohol, and dissected on a sterile Petri dish cover under 100 μl of Goodwin's isotonic IPL-52B tissue culture media (Goodwin and Adams, 1980).

Five to 10 eggs (2–4 and 18–24 hr post oviposition) were gathered from each host. Eggs were washed 3 times with media to remove the debris and hemolymph. A 100 μl, Drummond® pipette was used to transfer the eggs. In one case, a line drawing was made of a dissected ovary held in Goodwin's culture medium under a binocular microscope at 40×.

Measurement of egg volume

Eggs were held in Goodwin's tissue culture medium that had an osmolarity adjusted to be comparable with the host's hemolymph (0.358 osmolar, pH 7.5). Dimensions of eggs were measured under a dissecting microscope with an ocular grid. The volume of the parasitoid egg was estimated by using the formula for the volume of a cylinder.

Incorporation of [^{35}S]methionine for autoradiography

Dissected eggs (35–40 for one-dimensional and 115 for two-dimensional electrophoresis) were incubated in Goodwin's IPL-52B, minus yeastolate, leucine and methionine (K. C. Biological, Lenxa, KS, U.S.A.). Glutamine and leucine (2 mg/ml) each were added to IPL-52B and the medium sterilized by filtration using a Millipore® filter (Durapore®, 0.2 mm). Translation grade [^{35}S]L-methionine (New England Nuclear; sp. act. 1109 Ci/mmol) was added to each 100 μl sample using a 10 μl syringe to deliver 10 μl containing 100 μCi of ^{35}S-radiolabel. Eggs were incubated in the mixture for 2 hr at 24°C in a 100% r.h. chamber after which the radioactive medium was removed and the eggs were rinsed 3 times with distilled water.

Each sample of eggs was transferred to 100 μl of water in a microfuge tube using the positive displacement syringe. An equal volume of 10% TCA was added. The samples of eggs were disrupted by sonication for 1 min using a cuphorn sonicator (Model W-375, Heat Systems-Ultrasonics Inc.). The sample was centrifuged at 10,000 *g* and the supernatant discarded; the TCA precipitate was saved. Proteins were dissolved in 3% sodium dodecyl sulfate (SDS), 5% dithiothreitol (DDT), 100 μl 10 mM Tris buffer, pH 6.8 and boiled for 10 min. Bromophenol blue (1%) was incorporated in the buffer to serve as both a pH indicator and tracking dye during electrophoresis.

One-dimensional SDS gel electrophoresis

The Laemmli (1970) discontinuous electrophoresis system consisted of 12.5% polyacrylamide in 0.375 M Tris–HCl, pH 8.7, that contained 0.1% SDS in the running gel. All electrophoretic chemicals were from BioRad®. The stacking gel was 4% polyacrylamide in 0.125 M Tris–HCl, pH 6.7 and 0.1% SDS. Electrophoresis buffer consisted of Tris (30 g/l), glycerol (144 g/l) and SDS (5 g/l). Sample buffer was fortified with 2.5% SDS, 5% DTT, and bromophenol blue tracking dye. Samples were denatured by boiling in sample buffer (3% SDS, 5% DTT, 100 μM Tris buffer, pH 6.8 (TCA precipitate from 35–40 eggs) for 3 min. Bromophenol blue (1%) was incorporated in the buffer as both a pH indicator and tracking dye during electrophoresis. High and low molecular weight standards were treated as other protein samples. Conditions for electrophoresis were 15 mA until the tracking dye was 1–2 cm from the bottom of the 16 cm long, 1 mm thick gel (7–8 hr).

Two-dimensional gel electrophoresis

The basic two-dimensional gel was run according to O'Farrell (1975) with modifications described by Pollard (1982) and Laemmli (1970). The TCA precipitate, prepared as above, from 115 eggs was dissolved in 30–40 μl of lysis buffer (Pollard, 1982). The sample was then run on the first-dimension isoelectric focusing gel over the pH range of O'Farrell (1975). Second-dimension electrophoresis was in SDS/12.5% polyacrylamide gels as described for the one-dimensional SDS gel above according to Laemmli (1970).

Staining and autoradiography

The one-dimensional SDS gels were stained for 16 hr with 0.05% Coomassie Brilliant Blue® R-250 in 10% TCA, 45% methanol and 45% (v/v) water and then were dried and used to prepare autoradiograms. The two-dimensional gels were first stained with Coomassie Brilliant Blue R-250, counter-stained with silver (Wray *et al.*, 1981) and then dried.

Autoradiograms were prepared by exposing the dried gel to Ultrafim® (LKB) for 2–5 days at −70°C. The radioactivity of the TCA precipitated protein ranged from 300,000 to 400,000 cpm per lane in the one-dimensional gels and from 300,000 to 500,000 cpm in the two-dimensional gels. Films were developed in Kodak Dektol® and fixed with Kodak Rapid Fix.

Molecular weights were obtained from a linear regression analysis of the logarithm of molecular weight standards (Pharmacia® low molecular weight calibration kit) against their relative migration on a one-dimensional SDS gel (Poole and Borchers, 1979).

Incorporation of labeled leucine and uridine

Procedures used by Antley and Fox (1970) served as a basis for experiments that were conducted on each of the egg development stages. Each test (10 eggs) utilized 2 μCi of either [^{3}H]leucine (New England Nuclear; sp. act. 147 Ci/mmol) or [^{3}H]uridine (New England Nuclear; sp. act. 27.1 Ci/mmol) incubated in 50 μl of Goodwin's IPL-52B medium for 2 hr at 24°C. Eggs and media were transferred to microfuge tubes using a Drummond pipette. Bovine serum albumin (BSA) (14 μg) was added as a co-precipitate. An equal volume of 10% TCA was added, the eggs sonicated for 1 min, centrifuged, then washed 3 times with 10% TCA. The pellets were resuspended in 100 μl of 0.4 M Tris–HCl, pH 8.8 and transferred to 10 ml of Insta-Gel® (Packard) for liquid scintillation spectroscopy. Due to the limitations of measuring the minute quantities of protein in the TCA pellet, the incorporation of label was expressed on a per egg basis.

Inhibition of protein and RNS synthesis

Adapting the methods reported previously for sea urchin eggs (Cross and Cousineau, 1964), actinomycin-D (2 μg/ml) was used to inhibit the incorporation of 2 μCi of [^{3}H]uridine by ovarian eggs. Puromycin (40 μg/ml) was used to inhibit the incorporation of 2 μCi of [^{3}H]leucine.

Eggs were dissected from the reservoirs of two ovaries and incubated in 100 μl of IPL-52B minus leucine and fortified with 2 mg of glutamine. Incubation was for 16 hr at 24°C on a rotary shaker. TCA was added, the samples, sonicated for 1 min, and centrifuged for 5 min. Supernatant was removed and the pellet washed 3 times with 10% TCA. The protein pellet was added to 10 ml of Insta-Gel for liquid scintillation measurements.

Comparison of adult male and female wasp hemolymph

Hemolymph was collected from three ovipositing females and three males of *M. croceipes* 2–5 days after emergence. The hemolymph was collected by clasping the abdominal tip

with forceps and gently separating the internal organs from the abdomen under a 50 μl drop of buffer (0.125 M Tris–HCl, pH 6.7), thereby irrigating the hemocoel. Care was taken to ensure that the digestive tract was intact before proteins in the drop were analyzed by SDS–PAGE. Hemolymph from one individual wasp per drop was applied to each gel lane and the electrophoretic procedure carried out as described above.

RESULTS

Location of oocyte development

Each of the two ovaries of *M. croceipes* consists of a pair of ovarioles that contain the developing egg chambers (Fig. 1). Each egg chamber consists of an egg surrounded by a follicle layer and a cluster of attached nurse cells (Fig. 1A). Eggs in each ovariole develop synchronously. As the eggs move down the egg tube, absorption of the material in the nurse cells into the eggs is completed before they reach the egg reservoir. The follicle layer presumably is discarded from eggs in the reservoir (Fig. 1B) because two types of eggs were extruded from the open end of the reservoir, those with and those without the follicle layer. Some eggs could be seen in the process of shedding the follicle. However, eggs extruded from the calyx did not have a follicle layer but were surrounded only by a chorion (Fig. 1C).

Stages of embryo development

Oogenesis studies included three stages of egg development shown in Fig. 2A–C. the bi-lobed egg chambers were removed from the egg tubes (Fig. 2A). The reservoir eggs varied in their development with some being more immature, having the follicle layer attached (Fig. 2B), while others were more mature and without the follicle layer. Mature eggs, surrounded by a chorion, were taken from the calyx (Fig. 2C).

Embryogenesis studies included two stages of development (Fig. 2–D–E). Eggs were dissected from second instar larvae of *H. zea* within 3 hr of oviposition and were similar to the egg in Fig. 2D. Blastoderm formation was visible from 4 to 6 hr post-oviposition (Fig. 2D). After 24 hr, the embryo was surrounded by the trophaminon cellular membrane and the external, transport chorion (Fig. 2E). The embryo continued to grow inside the chorion and

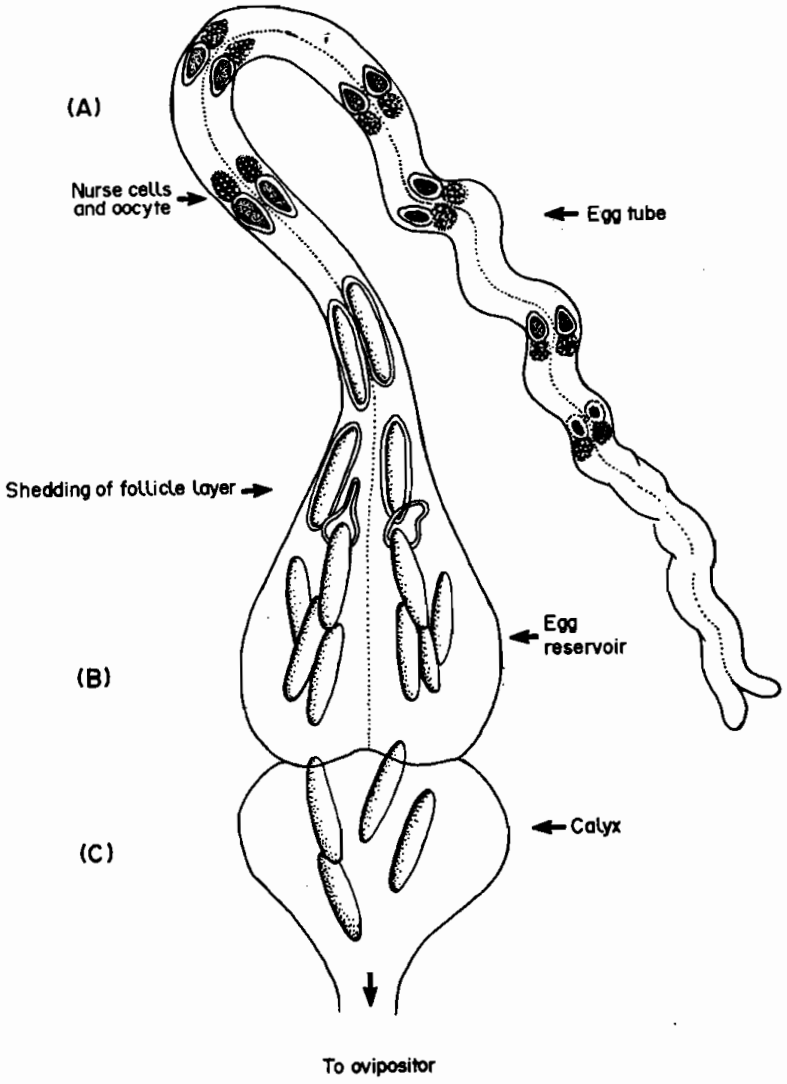

Fig. 1. Ovary of *M. croceipes* showing developmental sequence of egg formation: (A) egg tube; (B) reservoir eggs; (C) calyx eggs.

trophaminon (Fig. 2E) attaining a volume of 24 nl by 24 hr (eclosion occurred at 40 hr].

Autoradiograms of SDS–PAGE

Radioactive [^{35}S]methionine was used to label egg proteins for autoradiography before SDS–PAGE separation (Fig. 3). Each lane was qualitatively the same. Individual polypeptides differed in their relative intensity but appeared in all stages. The overall pattern of protein synthesis for oogenesis was similar to that for embryogenesis (Fig. 3A–C compared with D,E).

Synthesis of protein and RNA

Both total uptake and incorporation of [^{3}H]leucine into eggs was measured in triplicate at the five stages of egg development (Fig. 4). Stages of egg development were determined by their location in the egg tube, reservoir, and calyx. Eggs were also dissected from the host 4–6 hr post-oviposition (blastoderm formation), and 18–24 hr after initiation of embryogenesis. Linear regression (Poole and Borchers, 1979) of total (Y) vs incorporation (X) for 15 pairs of data was $Y = 209 + 0.83\,X$, $r = 0.97$. Therefore, incorporation was 83% of the total uptake.

Incorporation of tritiated leucine and uridine was measured at five stages of egg development during oogenesis and embryogenesis (Fig. 4). On a per egg basis, leucine incorporation remained relatively constant but then showed a major increase during late embryogenesis. Uridine incorporation declined at each consecutive stage until early embryogenesis then became elevated again during late embryogenesis.

The rate of labeled amino acid incorporation was probably influenced by the size of the embryo. The volume of the eggs used in measuring incorporation at the various stages in Fig. 4A-E ranged from 3.2 to 23 nl. After 4 hr of embryogenesis, the increased size of the eggs correlated with the radioactivity incorporated.

Measurements in Fig. 4 had a coefficient of variation of approximately 2%. This high degree of precision was achieved by using BSA as a coprecipitate.

Inhibition of tritiated uridine or leucine incorporation

Figure 5 illustrates inhibition of protein and RNA synthesis in the ovarian egg by puromycin and actinomycin, respectively. Although actinomycin-D inhibited RNA synthesis as expected, protein synthesis actually increased (Fig. 5A).

Two-dimensional electrophoresis

Since no qualitative differences were evident in protein patterns analyzed by one-dimensional SDS–PAGE, the two-dimensional electrophoretic technique of O'Farrell (1975) was used to further resolve the polypeptides.

Silver-stained 2D-gels were used to compare polypeptides in preblastoderm eggs (4–6 hr post-oviposition) (Fig. 6A) with polypeptides found in the same number of eggs during mid-embryogenesis (18–24 hr post-oviposition) (Fig. 6B). Thirty-seven major proteins were scored by tracing over prominant spots. Sixty-four spots were traced from the photo in Fig. 6B. The two tracings had 22 spots in common.

Newly synthesized polypeptides were detected by autoradiography (Fig. 6C) of the silver-stained gel (Fig. 6B). Fifty-nine spots were traced from the autoradiogram. Of those spots, 27 were in the same location as the silver-stained source, Fig. 6B. When transparent, color-coded overlays were used to compare the autoradiogram with the original preblastoderm pattern (Fig. 6A), 22 spots were in common.

Hemolymph comparison

Figure 7 is a silver-stained SDS–PAGE gel that compared female and male hemolymph proteins from adult *M. croceipes*. Each SDS gel lane represented the hemolymph from one insect. The purpose of this experiment was to identify vitellogenin as a reference protein. However, no sex-specific differences in major polypeptides were detected using this technique.

DISCUSSION

Eggs of *M. croceipes* develop in the two ovarioles of each ovary. Morphologically, they are polytrophic and do not appear to differ from free-living insects with the same type of oocyte development (Wigglesworth, 1950). Functionally, they may differ in not requiring maternal yolk proteins for development. Hemolymph proteins of ovipositing *M. croceipes* females did not appear different from hemolymph proteins of males on SDS–PAGE, indicating the absence of vitellogenin. Synthesis of vitellogenin by fat body and uptake of the protein by the ovaries is well documented in other insects (Kunkel and Nordin, 1985). Apparently the oocytes in *M. croceipes* synthesize required proteins from precursor molecules found in the host's hemolymph. The permeability of the egg chorion apparently is not a limiting factor since most of the [^{3}H]leucine taken up by the egg was incorporated (83%) and the egg continued to increase in size inside the chorion during its development. It was assumed from the results with [^{3}H]leucine that [^{3}H]uridine incorporation also would not be affected by chorion permeability.

The level of protein synthesis in eggs 2–4 hr after oviposition was relatively similar to that in oocytes found in the calyx, reservoir, and egg tubes of the ovarioles, indicating that synthesis was ongoing and was not initiated by the act of oviposition. Mechanical activation may be required to begin development (Went and Krause, 1974; Went, 1982) but not to start protein synthesis.

Incorporation of uridine during early oocyte development may be interpreted as synthesis of maternal RNA. A subsequent decline in RNA synthesis occurred as the oocyte matured. Apparently, the stable maternal transcripts were sufficient to support the protein synthesis required for egg maintenance and growth. Resumption of RNA synthesis occurred after blastoderm formation when expression of the embryonic genome took place. These results were similar to that of egg development in the chironomid midge, *Smittia* spp. (Jäckle and Kalthoff, 1979) and *D. melanogaster* (Meigen) (Ransom, 1982).

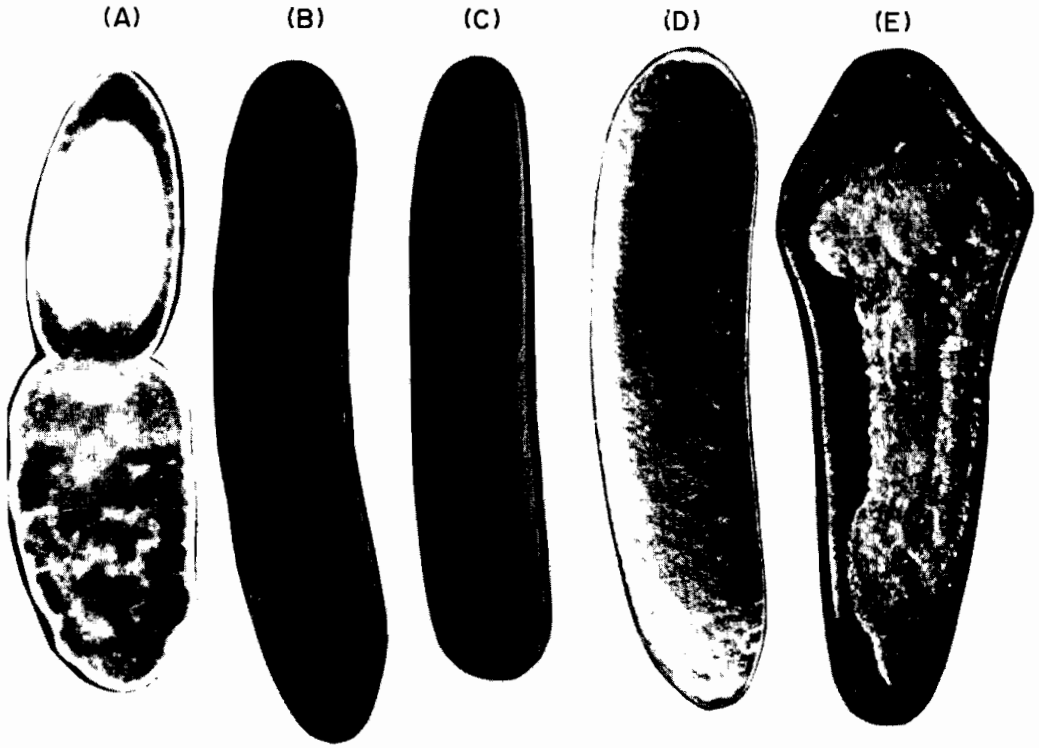

Fig. 2. Eggs dissected from (A) ovary egg tube (largest egg vol., 3 nl), (B) reservoir egg with follicle attached (egg vol., 3 nl), and (C) calyx (egg vol., 3 nl). Embryonic eggs were dissected from the host, *H. zea*, second instar larvae at (D) blastoderm formation 4–6 hr (egg vol., 5 nl), and (E) before egg hatch 18–24 hr after oviposition (egg vol. 24 nl).

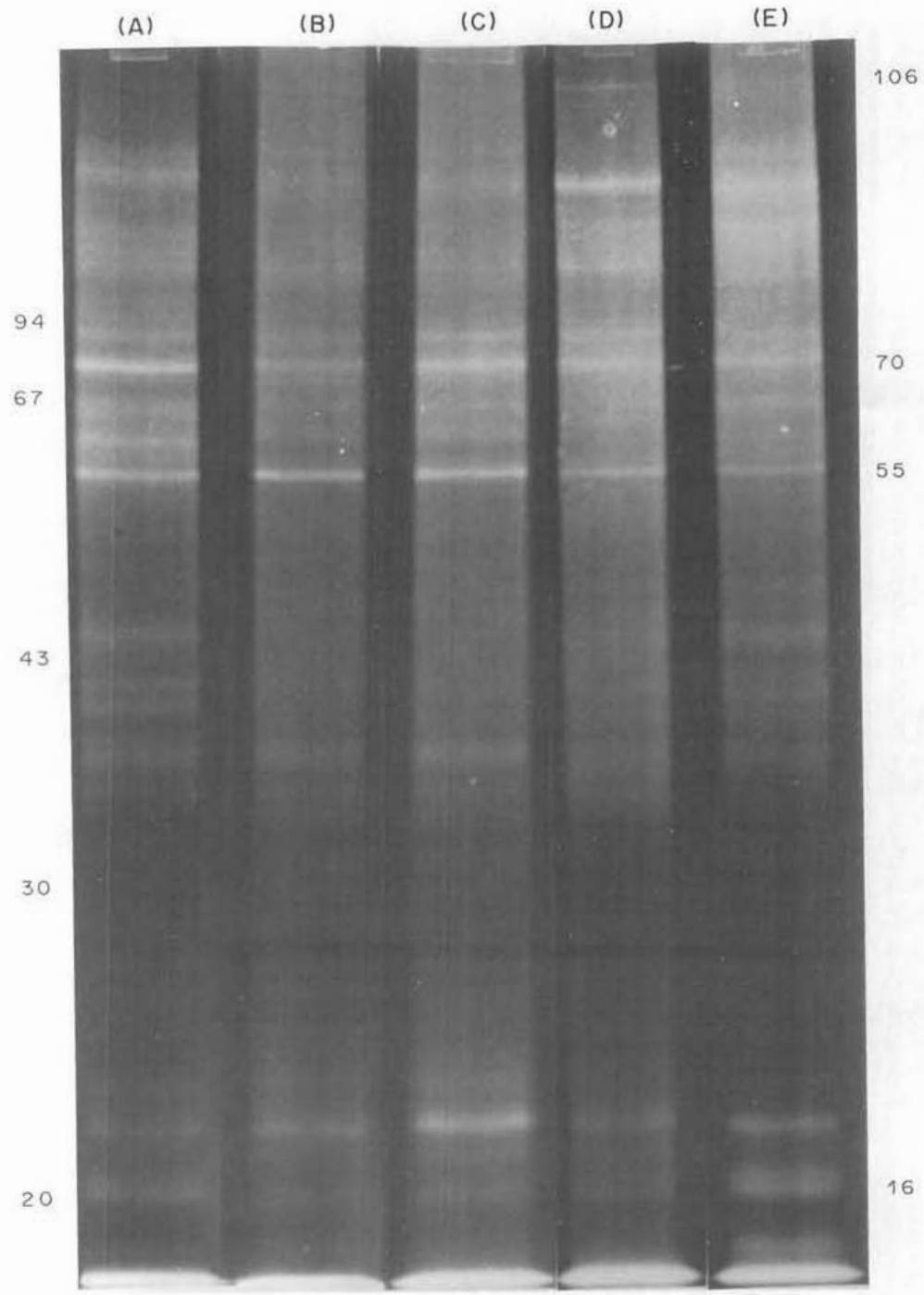

Fig. 3. Autoradiograms of SDS–PAGE. Proteins synthesized by the developing egg of *M. croceipes in vitro*. Eggs (30–45) from each stage were incubated in 100 μl of Goodwin's culture medium containing 10 μl of [^{35}S]L-methionine for 2 hr at 24°C. Exposures were adjusted to minimize differences between lanes. Each lane contained 300,000–400,000 cpm. Tubule eggs (A), reservoir eggs (B), calyx eggs (C), 4-hr oviposited eggs (D), and 18-hr oviposited eggs (E). Gels were calibrated with the following molecular weight markers (kDa): phosphorylase b, 94; bovine serum albumin, 67; ovalbumin, 43; carbonic anhydrase, 30; soybean trypsin inhibitor, 20. Approximate R_f values for several major egg proteins are on the right.

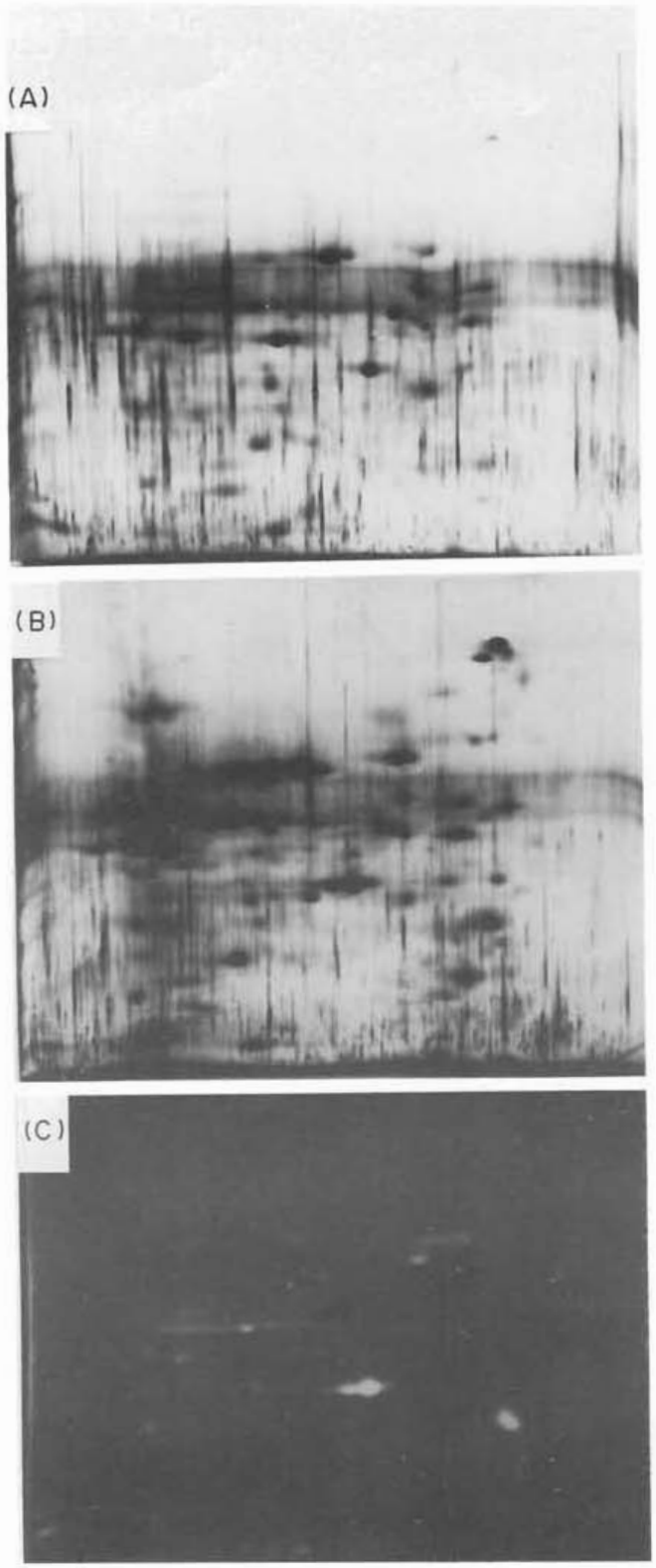

Fig. 6. Silver-stained two-dimensional gel of 115 eggs before blastoderm formation (4–6 hfr) (A), and after 18–24 hr (B). An autoradiogram of the silver-stained gel (C).

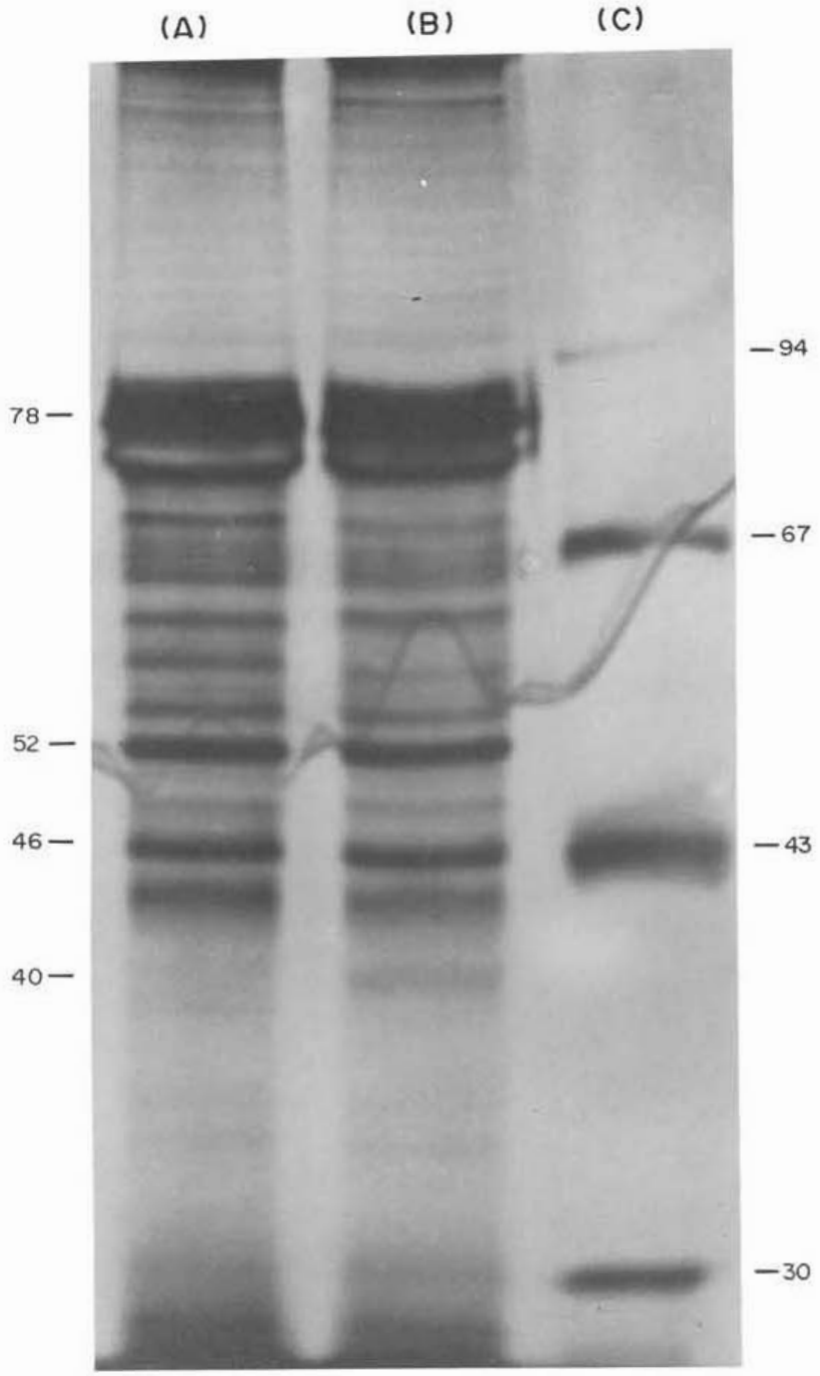

Fig. 7. Silver-stained SDS–PAGE analysis of male and female hemolymph from *M. croceipes*. Adult male (A), adult female (B), hemolymph and (C) molecular weight standards (kDa).

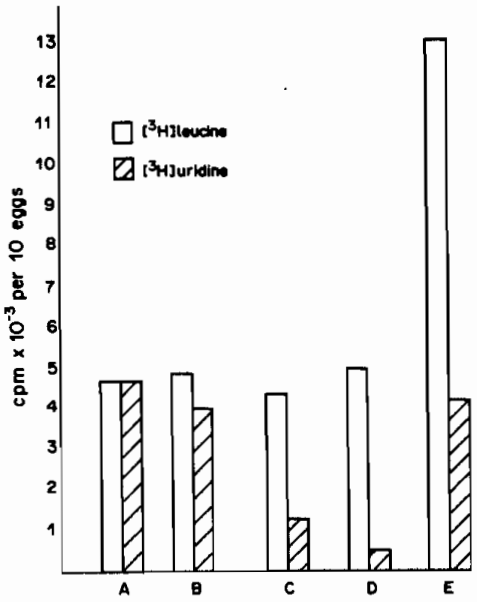

Fig. 4. Incorporation of tritiated leucine and uridine was measured at five egg-development stages, including oogenesis and embryogenesis (see Fig. 2 for legend).

Results of one-dimensional SDS–PAGE comparing protein synthesis during oogenesis with embryogenesis revealed that most of the polypeptides synthesized during oogenesis were similar to those found during embryogenesis. Only subtle changes in the intensity of certain bands were observed by one-dimensional SDS–PAGE even though obvious morphological changes in development had occurred. This constancy of proteins separated by one-dimensional SDS during egg development was also reported in early *D. melanogaster* (Meigen) embryogenesis (Roberts and Graziosi, 1977; Savoini *et al.*, 1981).

Although one-dimensional gels failed to detect changes in new polypeptides being synthesized as a correlate of egg development and morphology, two-dimensional (O'Farrell, 1975), silver-stained gels and autoradiography did detect changes in polypeptide synthesis. This demonstrates the greater resolving power of two-dimensional electrophoresis. The major polypeptides that dominated in one-dimensional gels were also evident in two-dimensional gels. Synthesis of new polypetides was evident when the two-dimensional gels of eggs before blastoderm formation were compared to those of eggs after blastoderm formation. These results with *M. croceipes* are consistent with those of other workers (Gutzeit and Gehring, 1979; Jäckle and Kalthoff, 1979; Sakoyama and Okubo, 1981; Trumbly and Jarry, 1983; Summers *et al.*, 1986) on *D. melanogaster* eggs. Trumbly and Jarry (1983), using two-dimensional gels, emphasized the increase in zygotic polypeptides evident after blastoderm but did not extend their observations beyond 8 hr. Summers *et al.* (1986) performed two-dimensional gels on all 15 stages of embryogenesis and described the changes in individual polypeptides as a "modulation" in rates of synthesis. Attempting to integrate our work with these and other workers (Cross and Cousineau, 1964—sea urchin eggs; Jäckle and Kalthoff, 1979; Jäckle, 1980—*D. melanogaster*), it seems reasonable that most major polypeptides in *M. croceipes* are structural proteins such as tubulin, myosin, keratin, and actin and would be expected to be present in all cells. Dominance of these structural proteins would explain why our one-dimensional gels have similar patterns from oogenesis through 18–24 hr of embryogenesis.

More work needs to be directed toward identifying the functions of the major polypeptides in insect eggs. Vitellin, another insect egg protein was of particular interest to this study. In insect eggs with yolk, vitellogenin is sex-specific, synthesized in the female fat body and converted into vitellin, the yolk protein (Kunkel and Nordin, 1985). Our findings indicate that, unlike insects that depend on yolk in the eggs as a nutritional source, *M. croceipes* may not have yolk proteins because we did not observe vitellin in female hemolymph. This possibility is consistent with possession of a thin, permeable chorion, metabolically active trophaminon (Ivanova-Kasas, 1972), and ability to incorporate amino acids from culture

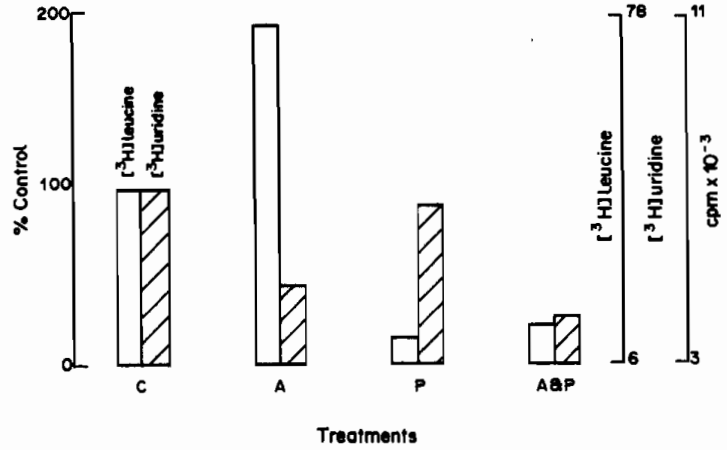

Fig. 5. The effect of actinomycin-D and/or puromycin on incorporation of tritiated leucine and tritiated uridine into reservoir eggs (Fig. 1B): (C) control eggs without treatment; (A) actinomycin-D treatment; (P) puromycin treatment; (A&P) combined drug treatment.

media. Other eggs of endoparasitic insect species may be adapted similarly.

Acknowledgements—Manuscript reviews by Drs J. Baker, D. Dahlman, K., Vick and P. Greany are appreciated. We acknowledge the technical assistance of Mr C. R. Dillard and Mrs E. Turner for typing the manuscript. Mention of a commercial or proprietary product does not constitute an endorsement by the USDA.

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